Use of Infrared Sensors for Early Detection of Bacterial Wilt Caused by *Ralstonia solanacearum* in Tomato Plants

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ABSTRACT

Infrared thermography can be used to detect water-stress induced temperature changes in plants. Bacterial wilt, a destructive disease of tomato caused by *Ralstonia solanacearum*, induces water stress in the host, leading to wilt and plant death. Fifty plants were inoculated with a bacterial suspension of 10^8 colony forming units (CFU) and fifty non-inoculated plants were maintained as healthy controls. Leaf temperature of inoculated and non-inoculated plants was measured with two infrared sensors, a low-cost infrared thermometer, and a thermal camera. The test was performed twice. In the first experiment, the incidence of bacterial wilt was 62%. Leaf temperature of healthy and diseased plants was similar for four days after inoculation. On the fifth and sixth days, leaf temperature of inoculated plants was 0,9 °C and 1,9 ºC higher, respectively, than the temperature of healthy plants. Wilt symptoms were first observed seven days after inoculation. In the second experiment, which coincided with cooler weather conditions (15 ºC during the day), disease incidence was 38%. Wilt symptoms were observed 10 days after inoculation, but temperature differences were observed seven days after inoculation. The use of this methodology allowed detection of differences in temperature two to three days before symptoms were visible. Application of this technology may facilitate management decisions for bacterial wilt.

Keywords: Infrared sensing, tomato wilt, *Ralstonia solanacearum*, infrared thermography, Costa Rica.

1. INTRODUCTION

R. solanacearum EF Smith , formerly called *Pseudomonas solanacearum* is a Gram-negative and aerobic plant pathogenic bacteria (Schell, 2000; Jones, 2001) in a very wide range of plant species, including tomato, chili, potato, and banana (Hayward, 2000). This soilborne pathogen invades the host through natural wounds that occur during the roots and root hairs emergence, through the lenticels, mechanical damage (Hernández et al.,2005) and nematodes wounds (Singh and Siddiqui, 2011).

Once inside, the bacteria colonizes the xylem, the population could be higher than 10^{10} CFU per centimeter of stem (Von Bodman et al., 2003). Later, the bacteria invade the phloem and cortex (Jones 2001). Despite being flagellated, their own mobility within the plant is limited (Tans-Kersten et al., 2001).

The first symptom is wilting of the youngest leaves during the hottest part of the day, and the field distribution of wilt disease usually occurs in patches. In soil, the bacterium has little mobility, depending on the splash and runoff of rain water (Tans-Kersten et al.,

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2001). Normally, diseased plants are diagnosed when the infection is advanced, this time of no visual symptoms alloy the bacterium dissemination (Swanson et al., 2005).

Chiwaki et al. (2005) found that asymptomatic infected tomato plants with *R. solanacearum* were 0.8 °C warmer than healthy plants. Further, differences were observed for up to 3 °C when comparing the temperature of infected plant relative to a healthy one.

Other similar investigation have shown that the canopy temperature is directly related to the water status of the plant, Stoll et al. (2008) used thermal imaging to monitor the pathogenesis of *Plasmopara viticola* in grape plants, concluding that thermography reveal the infection four days after inoculation and at least three days before the onset of symptoms.

Lindenthal et al. (2005) concluded that during the pathogenesis of downy mildew (*Pseudoperonospora cubensis*) on cucumber plants, this biotrophic fungus caused a progressive decrease in leaf temperature , showing 0.8 °C less than healthy leaves. In other hand, Wang et al. (2012) found that infection by *Fusarium oxysporum* f. sp. cucumerinum, causes decrease of the hydraulic and stomatal conductivity, and the loss its cooling capacity.

The objective of this study was to determine if infrared thermography can be used to detect disease-associated leaf temperature changes prior to symptom development in tomato plants infected with *R. solanacearum*.

2. MATERIALS AND METHODS

R. solanacearum strain was isolated from tomato plants showed the characteristic symptoms, the bacteria were transferred to tetrazolium medium (TZC) prepared according French et al. (1995). The identification of plant pathogenic bacteria was confirmed by PCR (polymerase chain reaction).

Stem inoculation was performed when plants were preflowering (50% of plants with visible flower buds). This occurred around day 26 after the plants were transplanted. The test was repeated twice in time, the first one during the month of October at the Experimental Station Baudrit Fabio Moreno, Alajuela, while the second took place during December in San Pedro, San José, both during 2012.

The experimental unit was a tomato plant and the test had two treatments: inoculated and healthy plants, each treatment had 50 replicates. Due to the greenhouse temperature varies in space and time, a paired set, which place two adjacent plants, was establish; each one treated differently but so close to each other, that would measure both at the same time. Potted plants were seeded at 0.2 m between plants and 0.4 m between pairs.

2.1 Measuring plant leaf temperature.

Leaf temperature of inoculated and non-inoculated plants was measured with two remote sensors, an infrared thermal camera and an infrared thermometer. The temperature on the adaxial face of the youngest fully-expanded leaf was measured between 8:15 and 9:00 am; the assessments began after inoculation and conclude until the symptoms of the plants were visible.

A Kintrex infrared thermometer, model IRT0424 (accuracy of ± 1ºC), was used. The emissivity of the apparatus was set to 0.97 .The thermal images were taken with a Flir SC620 -660 P640 (spectral response ranging from 7.5 to 13.5 µm). To analyze the data only plants that showed symptoms of bacterial wilt were taken. This means that plants remained healthy, were not taken into the statistical analysis.

2.2 Comparison between the two devices.

The leaf temperature measured with two infrared devices was compare. These measurements were evaluated on the same leaf and time.

3. RESULTS AND DISCUSSION

On the fifth day, leaf temperature of inoculated plants was 0.9 °C higher, respectively, than the temperature of healthy plants (Table 1). On the sixth day, the difference increased to 1.9 °C, although even plants showed any symptom (Figure 1). These results agree with Chiwaki et al. (2005) who found that infected tomato plants with *R. solanacearum* had a greater temperature 0.8 °C than healthy plants.

Table 1.Temperature differences between healthy and infected tomato plants with *Ralstonia solanacearum.*

	First spare repetition		**First spare repetition**	
DAI	T diference	Significance*	T diference	Significance*
1	0,00	A	0,03	A
2	0,05	A	0,00	A
3	0,13	A	0,12	A
4	0,46	A	0,10	A
5	0,91	B	0,06	A
6	1,90	B	0,27	A
7	Visual symptoms		0,58	B
8			0,92	B
9			1,42	B
10			Visual symptoms	

* Significance levels were used to accept or reject the null hypothesis that the difference in temperature between infected and healthy plants is zero for each assessment day, A accepts the null hypothesis. Fisher LSD test, different letters are significantly different ($p \leq 0.05$).

The leaf temperature increase gradually while *R. solanacearum* colonizes the xylem of the plant (French et al., 1995), causing a loss of the hydraulic conductivity (Genin and Denny, 2012) and thereby increases the leaf temperature (Figure 2).

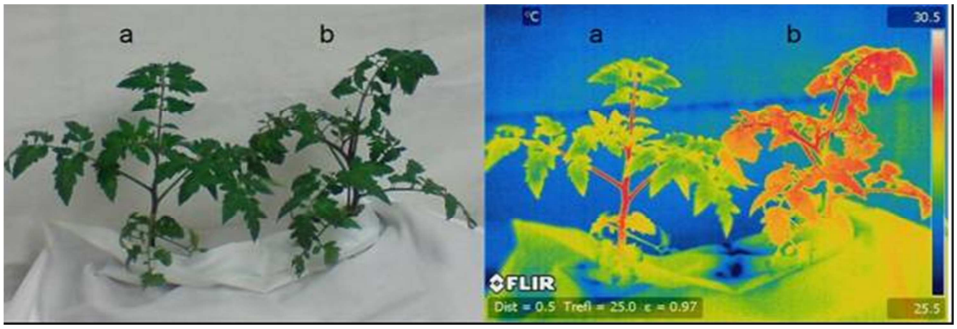

Figure 1. Digital color reflectance and thermal images of two tomato plants, healthy (a) and infected plant (b) with *R. solanacearum*, six days after inoculation.

One of the limitations for controlling the wilt disease is due to that the registered bactericides are systemic and basipetal, and depends on of the phloem. When symptoms appear, the plant has lost its vascular conductivity and agrochemicals can't move to the infection site. This occurs because the phloem conductivity is related with the water movement into the xylem (Hölttä and Nikinmaa, 2013); the sugar concentration of the source decreases the xylem water potential, generating the rise of water. Subsequently, the water passes into the phloem loading of sugars and later uploads into the sink (Berg, 2008). In other words, if the xylem flow stops the assimilation rate and the total of sugars decrease, sugars responsible for generating the osmotic differential movement to occur within the phloem (Villalobos et al., 2001).

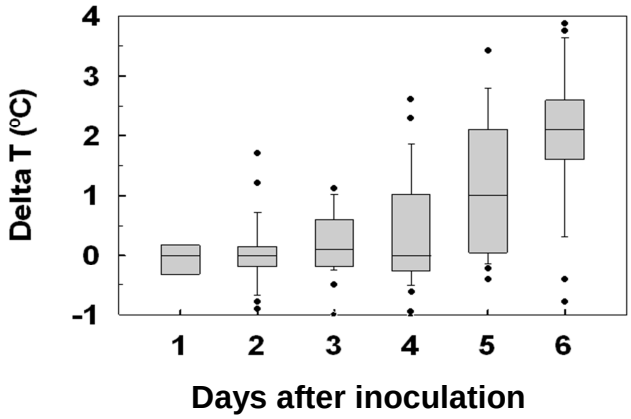

Figure 2. Differences in leaf temperature of plants infected with *R. solanacearum*, six days after inoculation.

During the second spare repetition, coincided with colder weather conditions, the difference in temperature between healthy and infected plants was not changed during the first 7 days after inoculation (Figure 3). The eighth day the infected pants showed no significant increase of 0.9 °C; finally, by the ninth day differences were 1.42 °C.

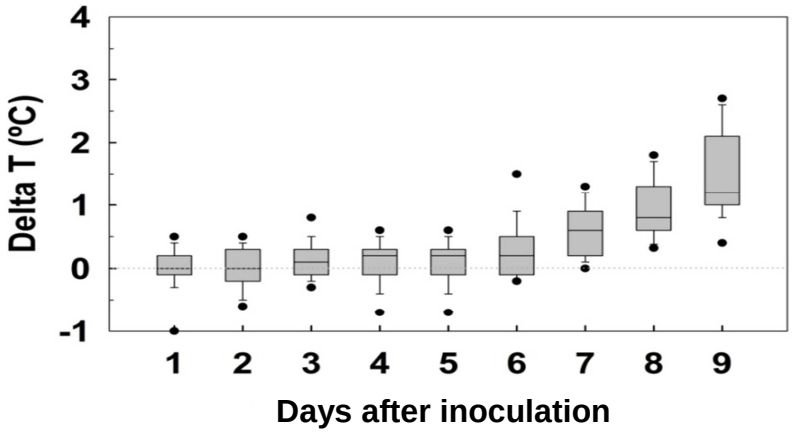

Figure 3. Differences in leaf temperature of plants infected with *R. solanacearum*, during the nine days after inoculation.

In studies of early disease detection with infrared technology, it was possible to demonstrate the infection of downy mildew on cucumber in advance 30% of the time before the visual appearance of symptoms (Lindenthal et al., 2005), 43% in *Plasmophara viticola* in grape (Stoll et al. 2008), 46% in the snuff mosaic virus (Chaerle et al,. 2004) and 55% of the *Fusarium oxysporum* in advance with cucumber leaves (Wang et al., 2012). In this work, we managed to detect temperature changes by 29% before the onset of symptoms.

Although differences were found between treatments, it should be noted that the change in leaf temperature depends on a number of abiotic factors such as the concentration of salts in the soil (Kang et al., 1991), drought, flooding or heat stress, among others, and can be altered by the action of pathogens such as *Fusarium* spp., which causes a water stress and therefore a rise in temperature (Wang et al,. 2012).

When compare the temperature data made with both devices, a between good relationship was found (Figure 4). It is possible to use the inexpensive infrared thermometer, also is easy to carry and can take the temperature quick and simple.

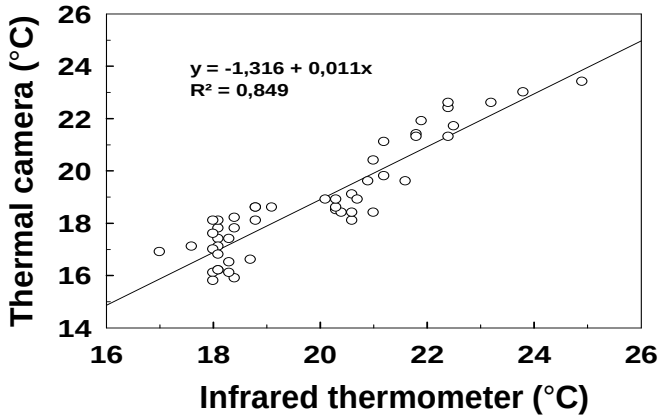

Figure 4. Correlation between leaf temperature measured thermographically and using the infrared thermometer.

Significant temperature increase was found two days before the onset of symptoms (Figure 2). The practical value is the possibility that, once identified plants with a higher temperature, could be excluded from pruning and de-suckering (Fortnum and Kluepfel, 2005), practices leading to spread of inoculum. In this case, discarding of plants is not an alternative, because the increase in temperature can have several causes.

If a production system where fertilization, the electrical conductivity of the substrate and dosage uniformity and irrigation is properly controlled, it would increase the likelihood that the temperature difference is caused by a pathogen infection is established. Under these conditions, plants can be marked and then discarded.

Furthermore, it is important that the thermographic data obtained are analyzed both temporally and spatially, which would identify whether the distribution of damage in the field corresponds to a biotic or abiotic problem (Agrios, 2005). Significantly, infection of plants under field conditions does not occur at the same time, but some plants presented symptoms while other infecting days and even weeks after.

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